



4176-101

**APPENDIX II**

**AFFIDAVIT BY DR. SUSAN DAGHER**

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**In re United States Patent Application of:**

**Applicant: CHILKOTI, ASHUTOSH**

**Application No.: 09/812,382**

**Date Filed: March 20, 2001**

**Title: FUSION PEPTIDES  
ISOLATABLY BY PHASE  
TRANSITION**

**Docket No.: 4176-101**

**Examiner: Walicka, M.A.**


**Art Group: 1652**

**Confirm. No.: 1286**

**23448**

**EXPRESS MAIL CERTIFICATE**

I hereby certify that I am mailing the attached documents to the Commissioner for Patents on March 1, 2004 in an envelope addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, and Express Mailed under the provisions of 37 CFR 1.10.

  
L. Stephen Lockett

March 1, 2004

Date

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**MAR 08 2004**

**AFFIDAVIT UNDER 37 C.F.R. §1.132 OF DR. SUSAN DAGHER  
IN U.S. PATENT APPLICATION NO. 09/812,383**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

I, **DR. SUSAN DAGHER**, being duly sworn, depose and say:

(1) THAT I have a PhD in molecular biology and am currently working as the primary research investigator for Phase BioScience, Inc (Chapel Hill, North Carolina), the licensee of U.S. Patent Application No. 09/812,383, filed on March 20, 2001 in the U.S. Patent and Trademark Office in my name for **“FUSION PEPTIDES ISOLATABLE BY PHASE TRANSITION”** (hereinafter “the Application”), which claims priority to U.S. Provisional Patent Application No. 60/190,659 filed March 20, 2000.

(2) THAT the Application discloses and claims fusion proteins that each comprise one or more target proteins of interest fused with one or more elastin-like polypeptides (ELPs) exhibiting inverse phase transition behavior, while such fusion proteins retain the inverse phase transition behavior of the ELPs and therefore can be isolated from other soluble proteins by inverse transition cycling (ITC) process (hereinafter “the Invention”).

(3) THAT in support of the Application, experiments have been conducted to show the use of various target proteins in forming ELP-containing fusion proteins and the inverse phase transition behavior exhibited by such fusion proteins. Specifically, thirty-six (36) ELP-containing fusion proteins were formed in *E. coli* by using known recombinant expression techniques consistent with the teachings and disclosures in Sections 5.4 and 6 of the Application.

(4) THAT the thirty-six ELP-containing fusion proteins comprised the combinations of:

- Insulin A peptide and ELP4-60 polypeptide with an enterokinase protease cleavage site therebetween;
- Insulin A peptide and ELP1-90 polypeptide with an enterokinase protease cleavage site therebetween;

- Insulin A peptide and ELP4-120 polypeptide with an enterokinase protease cleavage site therebetween;
- Insulin A peptide and ELP1-180 polypeptide with an enterokinase protease cleavage site therebetween;
- T20 peptide and ELP4-60 polypeptide with an enterokinase protease cleavage site therebetween;
- T20 peptide and ELP1-90 polypeptide with an enterokinase protease cleavage site therebetween;
- T20 peptide and ELP4-120 polypeptide with an enterokinase protease cleavage site therebetween;
- T20 peptide and ELP4-60 polypeptide with a thrombin protease cleavage site therebetween;
- T20 peptide and ELP1-90 polypeptide with a thrombin protease cleavage site therebetween;
- T20 peptide and ELP4-120 polypeptide with a thrombin protease cleavage site therebetween;
- T20 peptide and ELP4-60 polypeptide with a tobacco etch virus (TEV) protease cleavage site (cleavage between QS residues) therebetween;
- T20 peptide and ELP1-90 polypeptide with a TEV protease cleavage site (cleavage between QS residues) therebetween;
- T20 peptide and ELP4-120 polypeptide with a TEV protease cleavage site (cleavage between QS residues) therebetween;
- T20 peptide and ELP4-60 polypeptide with a TEV protease cleavage site (cleavage between QY residues) therebetween;
- T20 peptide and ELP1-90 polypeptide with a TEV protease cleavage site (cleavage between QY residues) therebetween;
- T20 peptide and ELP4-120 polypeptide with a TEV protease cleavage site (cleavage between QY residues) therebetween;

- Interferon alpha 2B protein and ELP1-90 polypeptide with a thrombin protease cleavage site therebetween;
- Tobacco etch virus protease and ELP4-60 polypeptide with a thrombin protease cleavage site therebetween;
- Tobacco etch virus protease and ELP1-90 polypeptide with a thrombin protease cleavage site therebetween;
- Tobacco etch virus protease and ELP4-120 polypeptide with a thrombin protease cleavage site therebetween;
- Tobacco etch virus protease and ELP1-180 polypeptide with a thrombin protease cleavage site therebetween;
- Small heterodimer partner orphan receptor and ELP1-90 polypeptide with a thrombin protease cleavage site therebetween;
- Androgen receptor ligand binding domain and ELP1-90 polypeptide with a thrombin protease cleavage site therebetween;
- Androgen receptor ligand binding domain and ELP1-180 polypeptide with a thrombin protease cleavage site therebetween;
- Glucocorticoid receptor ligand binding domain and ELP1-90 polypeptide with a thrombin protease cleavage site therebetween;
- Estrogen receptor ligand binding domain and ELP1-60 polypeptide with a thrombin protease cleavage site therebetween;
- Estrogen receptor ligand binding domain and ELP1-90 polypeptide with a thrombin protease cleavage site therebetween;
- Estrogen receptor ligand binding domain and ELP1-180 polypeptide with a thrombin protease cleavage site therebetween;

- Estrogen receptor ligand binding domain and ELP1-90 polypeptide with a TEV protease cleavage site (cleavage between QG residues) therebetween;
- G protein alpha Q and ELP1-90 polypeptide with a thrombin protease cleavage site therebetween;
- G protein alpha Q and ELP1-180 polypeptide with a thrombin protease cleavage site therebetween;
- 1-Deoxy-D-Xylulose 5-Phosphate reductoisomerase peptide and ELP1-60 polypeptide with a thrombin protease cleavage site therebetween;
- 1-Deoxy-D-Xylulose 5-Phosphate reductoisomerase peptide and ELP1-90 polypeptide with a thrombin protease cleavage site therebetween;
- 1-Deoxy-D-Xylulose 5-Phosphate reductoisomerase peptide and ELP1-180 polypeptide with a thrombin protease cleavage site therebetween;
- 1-Deoxy-D-Xylulose 5-Phosphate reductoisomerase peptide and ELP1-90 polypeptide with a TEV protease cleavage site (cleavage between QG residues) therebetween; and
- G protein alpha S and ELP1-90 polypeptide with a thrombin protease cleavage site therebetween.

(See Appendix A and Section D of Appendix B enclosed herewith)

(5) THAT a total of eleven (11) different target proteins were used for forming the ELP-containing fusion proteins as listed in paragraph (4) above, which included:

- (a) Insulin A peptide comprising 21 amino acid residues with molecular weight of about 2,400 Daltons;
- (b) T20 peptide comprising 36 amino acid residues with molecular weight of about 4,400 Daltons;

- (c) Interferon alpha 2B peptide comprising 188 amino acid residues with molecular weight of about 21,500 Daltons;
- (d) Tobacco etch virus protease comprising 242 amino acid residues with molecular weight of about 27,500 Daltons;
- (e) Small Heterodimer partner orphan receptor comprising 257 amino acid residues with molecular weight of about 28,000 Daltons;
- (f) Androgen receptor ligand binding domain comprising 258 amino acid residues with molecular weight of about 30,100 Daltons;
- (g) Glucocorticoid receptor ligand binding domain comprising 279 amino acid residues with molecular weight of about 32,100 Daltons;
- (h) Estrogen receptor ligand binding domain comprising 296 amino acid residues with molecular weight of about 33,200 Daltons;
- (i) G protein alpha Q comprising 359 amino acid residues with molecular weight of about 42,100 Daltons;
- (j) 1-Deoxy-D-Xylulose 5-Phosphate reductoisomerase peptide comprising 400 amino acid residues with molecular weight of about 43,5 Daltons; and
- (k) G protein alpha S comprising 380 amino acid residues with molecular weight of about 44,200 Daltons.

(See the target protein sequences in Section A of **Appendix B** enclosed herewith.)

(6) THAT the eleven target proteins as listed in paragraph (5) hereinabove are different in their respective:

- primary structures;
- secondary structures;
- tertiary structures;

- molecular weights;
- electric charge distributions;
- viscosity; and
- biological functions.

(7) THAT five (5) different elastin-like polypeptides (ELPs) were used for forming the fusion proteins as listed in paragraph (4) hereinabove. The sequences of such ELPs are disclosed in Section B of **Appendix B** enclosed herewith.

(8) THAT eleven (11) different spacer peptides containing various protease cleavage sites were used for joining the target proteins with the ELPs in forming the fusion proteins as listed in paragraph (4) hereinabove. The spacer peptide sequences are disclosed in Section C of **Appendix B** enclosed herewith.

(9) THAT all of the thirty-six ELP-containing fusion proteins as listed in paragraph (4) hereinabove retained the inverse phase transition behavior of the ELPs.

(10) THAT the ELP-containing fusion proteins as listed in paragraph (4) hereinabove were specifically isolated and purified by using inverse transition cycling (ITC) techniques, according to the following experimental procedure:

**(A) Isolation and Purification of Fusion Proteins Containing Insulin A Peptide (InsA)**

A single colony of *E. coli* strain BLR (DE3) (Novagen) containing the respective ELP-InsA fusion protein was inoculated into 5 ml CircleGrow (Q-BIOgene, San Diego, CA) supplemented with 100 µg/ml ampicillin (Sigma) and grown at 37°C with shaking at 250 rpm for 5 hours. The



5 ml culture was then inoculated into a 500 ml culture and allowed to grow at 25°C for 16 hours before inducing with 1 mM IPTG for 4 hours at 25°C. The culture was harvested and suspended in 40 ml 20 mM Tris-HCL pH 7.4, 50 mM NaCl, 1 mM DTT and 1 Complete EDTA free Protease inhibitor pellet (Roche, Indianapolis, IN). Cells were lysed by ultrasonic disruption on ice for 3 minutes, which consisted of 10 seconds bursts at 35% power separated by 30 second cooling down intervals. Cell debris was removed by centrifugation at 20,000g, 4°C for 30 minutes.

Inverse phase transition was induced by adding NaCl to the cell lysate at room temperature to achieve a final concentration of 1.0 M therein, followed by centrifugation at 20,000g for 15 minutes at room temperature. The resulting pellet contained the respective ELP-InsA fusion protein and non-specifically NaCl precipitated proteins.

The pellet was re-suspended in 40 ml ice-cold ml 20 mM Tris-HCL pH 7.4, 50 mM NaCl, 1 mM DTT and re-centrifuged at 20,000 g, 4°C for 15 minutes to remove the non-specifically NaCl precipitated proteins. The inverse transition cycle was repeated two additional times to increase the purity of the respective ELP-InsA fusion protein and reduce the final volume to 0.5 ml.

#### (B) Isolation and Purification of Fusion Proteins Containing T20 Peptide (T20)

A single colony of *E. coli* strain BLR (DE3) (Novagen) containing the respective ELP-T20 fusion protein was inoculated into 500 ml CircleGrow (Q-BIOgene, San Diego, CA) supplemented with 100 µg/ml ampicillin (Sigma) and grown at 37°C with shaking at 250 rpm for 24 hours. The culture was harvested and suspended in 40 ml 50 mM Tris pH 8.0, 0.5 mM EDTA and 1 Complete Protease inhibitor pellet (Roche, Indianapolis, IN). Cells were lysed by ultrasonic disruption on ice for 3 minutes, which consisted of 10 seconds bursts at 35% power

separated by 30 second cooling down intervals. Cell debris was removed by centrifugation at 20,000g, 4°C for 30 minutes.

Inverse phase transition was induced by adding NaCl to the cell lysate at room temperature to achieve a final concentration of 1.0 M therein, followed by centrifugation at 20,000g for 15 minutes at room temperature. The resulting pellet contained the respective ELP-T20 fusion protein and non-specifically NaCl precipitated proteins.

The pellet was re-suspended in 40 ml ice-cold ml 50 mM Tris pH 8.0, 0.5 mM EDTA and re-centrifuged at 20,000 g, 4°C for 15 minutes to remove the non-specifically NaCl precipitated proteins. The inverse transition cycle was repeated two additional times to increase the purity of the respective ELP-T20 fusion protein and reduce the final volume to 5 ml.

#### (C) Isolation and Purification of Fusion Protein Containing Interferon Alpha 2B Peptide (IFNA2)

A single colony of *E. coli* strain BL21(DE3) TrxB<sup>-</sup> (Novagen) containing the ELP-IFNA2 fusion protein and Codon Plus-RIL plasmid (Stratagene) was inoculated into 500 ml CircleGrow (Q-BIOgene, San Diego, CA) supplemented with 100 µg/ml ampicillin (Sigma), 25 µg/ml Chloramphenicol (Sigma) and incubated at 27°C with shaking at 250 rpm for 48 hours. The culture was harvested and suspended in 50 mM Tris-HCL pH 7.4, 50 mM NaCl and 1 Complete EDTA free Protease inhibitor pellet (Roche, Indianapolis, IN). Cells were lysed by ultrasonic disruption on ice for 3 minutes, which consists of 10 seconds bursts at 35% power separated by 30 second cooling down intervals. Cell debris was removed by centrifugation at 20,000g, 4°C for 30 minutes.

Inverse phase transition was induced by adding NaCl to the cell lysate at room temperature to achieve a final concentration of 1.5 M, followed by centrifugation at 20,000g for 15 minutes at room temperature. The resulting pellet contained the ELP-IFNA2 fusion protein and non-specifically NaCl precipitated proteins.

The pellet was re-suspended in 40 ml ice-cold 50 mM Tris-HCL pH 7.4 and 50 mM NaCl and re-centrifuged at 20,000 g, 4°C for 15 minutes to remove the non-specifically NaCl precipitated proteins. The inverse transition cycle was repeated two additional times to increase the purity of the ELP-IFNA2 fusion protein and reduce the final volume to 5 ml.

#### (D) Isolation and Purification of Fusion Proteins Containing Tobacco Etch Virus Protease (TEV)

A single colony of *E. coli* strain BL21 star or BRL(DE3) containing pET15b-SD5-ELP-TEV constructs and Codon Plus-RIL plasmid (Stratagene) was inoculated into 500 ml CircleGrow (Q-BIOgene, San Diego, CA) supplemented with 100 µg/ml ampicillin (Sigma), 25 ug/ml Chloramphenicol (Sigma) and incubated at 27°C with shaking at 250 rpm for 48 hours. The culture was harvested and suspended in 50 mM Tris-HCL pH 8.0, 1 mM EDTA, 5 mM DTT, 10% glycerol and 1mM PMSF. Cells were lysed by ultrasonic disruption on ice for 3 minutes, consisting of 10 seconds bursts at 35% power separated by 30 second cooling down intervals. Cell debris was removed by centrifugation at 20,000g, 4°C for 30 minutes.

Inverse phase transition was induced by adding NaCl to the cell lysate at room temperature to achieve a final concentration of 1.5 M, followed by centrifugation at 20,000g for 15 minutes at room temperature. The resulting pellet contained the respective ELP-TEV fusion protein and non-specifically NaCl precipitated proteins.

The pellet was re-suspended in 40 ml ice-cold 50 mM Tris-HCL pH 8.0, 1 mM EDTA, 5 mM DTT, 10% glycerol and re-centrifuged at 20,000 g, 4°C for 15 minutes to remove the non-specifically NaCl precipitated proteins. The inverse transition cycle was repeated two additional times to increase the purity of the respective ELP-TEV fusion protein and reduce the final volume to 1 ml.

#### (D) Isolation and Purification of Fusion Protein Containing Small Heterodimer Partner Orphan Receptor (SHP)

A single colony of *E. coli* strain BL21 Star (DE3) containing the ELP-SHP fusion protein was inoculated into 500 ml CircleGrow (Q-BIOgene, San Diego, CA) supplemented with 100 µg/ml ampicillin (Sigma) and 10% sucrose and grown at 27°C with shaking at 250 rpm for 48 hours. The culture was harvested and suspended in 50 mM Tris-HCL pH 8.0, 150 mM KCL, 1 mM DTT 1 mM EDTA and 1 Complete EDTA free Protease inhibitor pellet (Roche, Indianapolis, IN). Cells were lysed by ultrasonic disruption on ice for 3 minutes, which consists of 10 seconds bursts at 35% power separated by 30 second cooling down intervals. DNA and RNA in the soluble lysate were further degraded by adding 2 µl Benzonase (Novagen) and incubating at 4°C for 30 minutes. Cell debris was removed by centrifugation at 20,000g, 4°C for 30 minutes.

Inverse phase transition was induced by adding NaCl to the cell lysate at room temperature to achieve a final concentration of 1.5 M, followed by centrifugation at 20,000g for 15 minutes at room temperature. The resulting pellet contained the ELP-SHP fusion protein and non-specifically NaCl precipitated proteins.

The pellet was re-suspended in 40 ml ice-cold 50 mM Tris-HCL pH 8.0, 150 mM KCL, 1 mM DTT 1 mM EDTA, and 1% N-Octylglucoside and re-centrifuged at 20,000 g, 4°C for 15 minutes

to remove non-specific insoluble proteins. The temperature transition cycle was repeated two additional times to increase the purity of the ELP-SHP fusion protein and reduce the final volume to 2 ml.

#### (F) Isolation and Purification of Fusion Proteins Containing Androgen Receptor Ligand Binding Domain (AR-LBD)

A single colony of *E. coli* strain BL21 Star (DE3) containing the respective ELP-AR-LBD fusion protein was inoculated into 500 ml CircleGrow (Q-BIOgene, San Diego, CA) supplemented with 100 µg/ml ampicillin (Sigma) and 10 µM DHT and grown at 27°C with shaking at 250 rpm for 48 hours. The culture was harvested and suspended in 40 ml 50mM Hepes pH 7.5, 150 mM NaCl, 0.1% N-Octylglycoside, 10% glycerol, 1 mM DTT, 1 µM DHT and 1 Complete EDTA free Protease inhibitor pellet (Roche, Indianapolis, IN). Cells were lysed by ultrasonic disruption on ice for 3 minutes, which consisted of 10 seconds bursts at 35% power separated by 30 second cooling down intervals. DNA and RNA in the soluble sonicate were further degraded by adding 2 µl Benzonase (Novagen) and incubating at 4°C for 30 minutes. Cell debris was removed by centrifugation at 20,000g, 4°C for 30 minutes.

Inverse phase transition was induced by adding NaCl to the cell lysate at room temperature to achieve a final concentration of 2.0 M, followed by centrifugation at 20,000g for 15 minutes at room temperature. The resulting pellet contained the respective ELP-AR-LBD fusion protein and non-specifically NaCl precipitated proteins.

The pellet was re-suspended in 40 ml ice-cold 50mM Hepes pH 7.5, 150 mM NaCl, 0.1% N-Octylglycoside, 10% glycerol, 1 mM DTT and 1 µM DHT and re-centrifuged at 20,000 g, 4°C for 15 minutes to remove the non-specifically NaCl precipitated proteins. The inverse transition

cycle was repeated two additional times to increase the purity of the respective ELP-AR-LBD fusion protein and reduce the final volume to 25 ml.

#### (G) Isolation and Purification of Fusion Protein Containing Glucocorticoid Receptor Ligand Binding Domain (GR-LBD)

A single colony of *E. coli* strain BL21 Star (DE3) containing the ELP-GR-LBD fusion protein was inoculated into 500 ml CircleGrow (Q-BIOgene, San Diego, CA) supplemented with 100 µg/ml ampicillin (Sigma) and grown at 37°C with shaking at 250 rpm for 24 hours. The culture was harvested and suspended in 50 mM Hepes pH 7.5, 150 mM NaCl, 1 mM DTT, 10% glycerol, 0.1% CHAPS and 1 Complete EDTA free Protease inhibitor pellet (Roche, Indianapolis, IN). Cells were lysed by ultrasonic disruption on ice for 3 minutes, which consisted of 10 seconds bursts at 35% power separated by 30 second cooling down intervals. DNA and RNA in the soluble lysate were further degraded by adding 2µl Benzonase (Novagen) and incubating at 4°C for 30 minutes. Cell debris was removed by centrifugation at 20,000g, 4°C for 30 minutes.

Inverse phase transition was induced by adding NaCl to the cell lysate at room temperature to achieve a final concentration of 2.0 M, followed by centrifugation at 20,000g for 15 minutes at room temperature. The resulting pellet contained the ELP-GR-LBD fusion protein and non-specifically NaCl precipitated proteins.

The pellet was re-suspended in 40 ml ice-cold in 50 mM Hepes pH 7.5, 150 mM NaCl, 1 mM DTT, 10% glycerol, 0.1% CHAPS and re-centrifuged at 20,000 g, 4°C for 15 minutes to remove the non-specifically NaCl precipitated proteins. The inverse transition cycle was repeated two

additional times to increase the purity of the ELP-GR-LBD fusion protein and reduce the final volume to 10 ml.

#### (H) Isolation and Purification of Fusion Proteins Containing Estrogen Receptor Ligand Binding Domain (ER $\alpha$ -LBD)

A single colony of *E. coli* strain BL21 Star (DE3) containing the respective ELP-ER $\alpha$ -LBD fusion protein was inoculated into 500 ml CircleGrow (Q-BIOgene, San Diego, CA) supplemented with 100  $\mu$ g/ml ampicillin (Sigma), 10% sucrose (Sigma) and grown at 27°C with shaking at 250 rpm for 48 hours. The culture was harvested and suspended in 40 ml 50mM Tris-HCL pH 8.0, 150 mM KCL, 1 mM EDTA, 1 mM DTT and 1 Complete EDTA free Protease inhibitor pellet (Roche, Indianapolis, IN). Cells were lysed by ultrasonic disruption on ice for 3 minutes, which consisted of 10 seconds bursts at 35% power separated by 30 second cooling down intervals. DNA and RNA in the soluble lysate were further degraded by adding 2  $\mu$ l Benzonase (Novagen) and incubating at 4°C for 30 minutes. Cell debris was removed by centrifugation at 20,000g, 4°C for 30 minutes.

Inverse phase transition was induced by adding NaCl to the cell lysate at room temperature to achieve a final concentration of 1.5 M, followed by centrifugation at 20,000g for 15 minutes at room temperature. The resulting pellet contained the respective ELP-ER $\alpha$ -LBD fusion protein and non-specifically NaCl precipitated proteins.

The pellet was re-suspended in 40 ml ice-cold 50mM Tris-HCL pH 8.0, 150 mM KCL, 1 mM EDTA, 1 mM DTT and re-centrifuged at 20,000 g, 4°C for 15 minutes to remove the non-specifically NaCl precipitated proteins. The inverse transition cycle was repeated two additional

times to increase the purity of the respective ELP-ER $\alpha$ -LBD fusion protein and reduce the final volume to 10 ml.

#### (I) Isolation and Purification of Fusion Proteins Containing G Protein Alpha Q (G $\alpha$ q)

A single colony of *E. coli* strain BL21 Star (DE3) containing the respective ELP-G $\alpha$ q fusion protein was inoculated into 500 ml CircleGrow (Q-BIOgene, San Diego, CA) supplemented with 100  $\mu$ g/ml ampicillin (Sigma) and 1  $\mu$ M GDP and grown at 37°C with shaking at 250 rpm for 24 hours. The culture was harvested and suspended in 40 ml 50mM Hepes pH 7.5, 150 mM NaCl, 1.0% CHAPS, 10% glycerol, 1 mM DTT, 10  $\mu$ M GDP and 1 Complete EDTA free Protease inhibitor pellet (Roche, Indianapolis, IN). Cells were lysed by ultrasonic disruption on ice for 3 minutes, which consisted of 10 seconds bursts at 35% power separated by 30 second cooling down intervals. DNA and RNA in the soluble lysate were further degraded by adding 2  $\mu$ l Benzonase (Novagen) and incubating at 4°C for 30 minutes. Cell debris was removed by centrifugation at 20,000g, 4°C for 30 minutes.

Inverse phase transition was induced by adding NaCl to the cell lysate at room temperature to achieve a final concentration of 2.0 M, followed by centrifugation at 20,000g for 15 minutes at room temperature. The resulting pellet contained the respective ELP-G $\alpha$ q fusion protein and non-specifically NaCl precipitated proteins.

The pellet was re-suspended in 30 ml ice-cold 50mM Hepes pH 7.5, 150 mM NaCl, 1.0% CHAPS, 10% glycerol, 1 mM DTT, 10  $\mu$ M GDP and re-centrifuged at 20,000 g, 4°C for 15 minutes to remove the non-specifically NaCl precipitated proteins. The inverse transition cycle



was repeated two additional times to increase the purity of the respective ELP-G<sub>αq</sub> fusion protein and reduce the final volume to 5 ml.

(J) Isolation and Purification of Fusion Proteins Containing 1-Deoxy-D-Xylulose 5-Phosphate Reductoisomerase (DXR)

A single colony of *E. coli* strain BL21 Star (DE3) containing the respective ELP-DXR fusion protein was inoculated into 500 ml CircleGrow (Q-BIOgene, San Diego, CA) supplemented with 100 µg/ml ampicillin (Sigma), 1mM MnCl<sub>2</sub> (VWR) and grown at 37°C with shaking at 250 rpm for 24 hours. The culture was harvested and suspended in 40 ml 0.1M Tris pH 7.6, 1 mM DTT and 1 Complete EDTA free Protease inhibitor pellet (Roche, Indianapolis, IN). Cells were lysed by ultrasonic disruption on ice for 3 minutes, which consisted of 10 seconds bursts at 35% power separated by 30 second cooling down intervals. DNA and RNA in the soluble lysate were further degraded by adding 2 µl Benzonase (Novagen) and incubating at 4°C for 30 minutes. Cell debris was removed by centrifugation at 20,000g at 4°C for 30 minutes.

Inverse phase transition was induced by adding NaCl to the cell lysate at room temperature to achieve a final concentration of 2.0 M, followed by centrifugation at 20,000g for 15 minutes at room temperature. The resulting pellet contained the respective ELP-DXR fusion protein and non-specifically NaCl precipitated proteins.

The pellet was re-suspended in 20 ml ice-cold 0.1 M Tris pH7.6, 1mM DTT and centrifuged at 20,000 g, 4°C for 15 minutes to remove the non-specifically NaCl precipitated proteins. The inverse transition cycle was repeated two additional times to increase the purity of the respective ELP-DXR fusion protein and reduce the final volume to 5 ml.

#### (K) Isolation and Purification of Fusion Protein Containing G Protein Alpha S (G $\alpha$ S)

A single colony of *E. coli* strain BL21 Star (DE3) containing the ELP-G $\alpha$ S fusion protein was inoculated into 500 ml CircleGrow (Q-BIOgene, San Diego, CA) supplemented with 100  $\mu$ g/ml ampicillin (Sigma) and grown at 37°C with shaking at 250 rpm for 24 hours. The culture was harvested and suspended in 40 ml PBS, 10% glycerol, 1 mM DTT and 1 Complete EDTA free Protease inhibitor pellet (Roche, Indianapolis, IN). Cells were lysed by ultrasonic disruption on ice for 3 minutes, which consisted of 10 seconds bursts at 35% power separated by 30 second cooling down intervals. DNA and RNA in the soluble lysate were further degraded by adding 2  $\mu$ l Benzonase (Novagen) and incubating at 4°C for 30 minutes. Cell debris was removed by centrifugation at 20,000g, 4°C for 30 minutes.

Inverse phase transition was induced by adding NaCl to the cell lysate at room temperature to a final concentration of 1.5 M, followed by centrifugation at 20,000g for 15 minutes at room temperature. The resulting pellet contained the ELP-G $\alpha$ S fusion protein and non-specifically NaCl precipitated proteins.

The pellet was re-suspended in 10 ml ice-cold PBS, 10% glycerol, 1 mM DTT and centrifuged at 20,000 g, 4°C for 15 minutes to remove the non-specifically NaCl precipitated proteins. The inverse transition cycle was repeated two additional times to increase the purity of the ELP-G $\alpha$ S fusion protein and reduce the final volume to 1 ml.

(11) THAT variations of the above-listed target proteins in their primary structures, secondary structures, tertiary structures, molecular weights, electrical charge distributions, viscosity, and biological functions,

did not prohibit the respective ELP-containing fusion proteins from retaining the inverse phase transition behavior of the ELPs.

Sue Dagher  
Dr. Susan Dagher

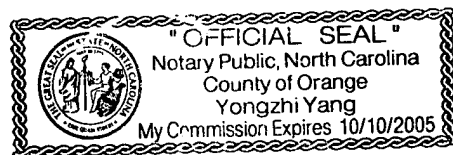
2-27-04  
Date

*Dr. Susan Dagher appeared before me on this 27 day of February, 2004. She declared to me that she is the person described in this Affidavit, and she executed this Affidavit before me, and declared that her execution was completely voluntary.*

State of North Carolina

County of Orange

(SEAL)



[Signature]  
Notary Public

My commission expires: 10/10/2005

## Appendix A: Fusion Proteins

FP #	ELP Construct			Protease Cleavage Site in Spacer	Target Protein Construct		
	ELP Identification	Amino Acid #	Molecular Weight		Target Peptide/Protein	Amino Acid #	Molecular Weight
1	ELP4-60	301	24.6 KDa	Enterokinase protease (DDDDK)	Insulin A Peptide	21	2.4 KDa
2	ELP1-90	451	35.2 KDa	Enterokinase protease (DDDDK)	Insulin A Peptide	21	2.4 KDa
3	ELP4-120	601	49.2 KDa	Enterokinase protease (DDDDK)	Insulin A Peptide	21	2.4 KDa
4	ELP1-180	901	70.4 KDa	Enterokinase protease (DDDDK)	Insulin A Peptide	21	2.4 KDa
5	ELP4-60	301	24.6 KDa	Enterokinase protease (DDDDK)	T20 Peptide	36	4.4 KDa
6	ELP1-90	451	35.2 KDa	Enterokinase protease (DDDDK)	T20 Peptide	36	4.4 KDa
7	ELP4-120	601	49.2 KDa	Enterokinase protease (DDDDK)	T20 Peptide	36	4.4 KDa
8	ELP4-60	301	24.6 KDa	Thrombin protease (R/G)	T20 Peptide	36	4.4 KDa
9	ELP1-90	451	35.2 KDa	Thrombin protease (R/G)	T20 Peptide	36	4.4 KDa
10	ELP4-120	601	49.2 KDa	Thrombin protease (R/G)	T20 Peptide	36	4.4 KDa
11	ELP4-60	301	24.6 KDa	TEV protease (Q/S)	T20 Peptide	36	4.4 KDa
12	ELP1-90	451	35.2 KDa	TEV protease (Q/S)	T20 Peptide	36	4.4 KDa
13	ELP4-120	601	49.2 KDa	TEV protease (Q/S)	T20 Peptide	36	4.4 KDa
14	ELP4-60	301	24.6 KDa	TEV protease (Q/Y)	T20 Peptide	36	4.4 KDa
15	ELP1-90	451	35.2 KDa	TEV protease (Q/Y)	T20 Peptide	36	4.4 KDa
16	ELP4-120	601	49.2 KDa	TEV protease (Q/Y)	T20 Peptide	36	4.4 KDa
17	ELP1-90	451	35.2 KDa	Thrombin protease (R/G)	Interferon Alpha 2B	188	21.5 KDa
18	ELP4-60	301	24.6 KDa	Thrombin protease (R/G)	Tobacco Etch Virus Protease	242	27.5 KDa
19	ELP1-90	451	35.2 KDa	Thrombin protease (R/G)	Tobacco Etch Virus Protease	242	27.5 KDa
20	ELP4-120	601	49.2 KDa	Thrombin protease (R/G)	Tobacco Etch Virus Protease	242	27.5 KDa
21	ELP1-180	901	70.4 KDa	Thrombin protease (R/G)	Tobacco Etch Virus Protease	242	27.5 KDa
22	ELP1-90	451	35.2 KDa	Thrombin protease (R/G)	Small Heterodimer Partner Orphan Receptor	257	28.0 KDa
23	ELP1-90	451	35.2 KDa	Thrombin protease (R/G)	Androgen Receptor Ligand Binding Domain	258	30.1 KDa

24	ELP1-180	901	70.4 KDa	Thrombin protease (R/G)	Androgen Receptor Ligand Binding Domain	258	30.1 KDa
25	ELP1-90	451	35.2 KDa	Thrombin protease (R/G)	Glucocorticoid Receptor Ligand Binding Domain	279	32.1 KDa
26	ELP1-60	301	23.5 KDa	Thrombin protease (R/G)	Estrogen Receptor Ligand Binding Domain	296	33.2 KDa
27	ELP1-90	451	35.2 KDa	Thrombin protease (R/G)	Estrogen Receptor Ligand Binding Domain	296	33.2 KDa
28	ELP1-180	901	70.4 KDa	Thrombin protease (R/G)	Estrogen Receptor Ligand Binding Domain	296	33.2 KDa
29	ELP1-90	451	35.2 KDa	TEV protease (Q/G)	Estrogen Receptor Ligand Binding Domain	296	33.2 KDa
30	ELP1-90	451	35.2 KDa	Thrombin protease (R/G)	G Protein Alpha Q	359	42.1 KDa
31	ELP1-180	901	70.4 KDa	Thrombin protease (R/G)	G Protein Alpha Q	359	42.1 KDa
32	ELP1-60	301	23.5 KDa	Thrombin protease (R/G)	1-Deoxy-D-Xylulose 5-Phosphate Reductoisomerase Peptide	400	43.5 KDa
33	ELP1-90	451	35.2 KDa	Thrombin protease (R/G)	1-Deoxy-D-Xylulose 5-Phosphate Reductoisomerase Peptide	400	43.5 KDa
34	ELP1-180	901	70.4 KDa	Thrombin protease (R/G)	1-Deoxy-D-Xylulose 5-Phosphate Reductoisomerase Peptide	400	43.5 KDa
35	ELP1-90	451	35.2 KDa	TEV protease (Q/G)	1-Deoxy-D-Xylulose 5-Phosphate Reductoisomerase Peptide	400	43.5 KDa
36	ELP1-90	451	35.2 KDa	Thrombin protease (R/G)	G Protein Alpha S	380	44.2 KDa

## **Appendix B: Protein Sequences**

### **(A). Target Protein Sequences**

#### **(1) Insulin A Peptide:**

GIVEQCCTSICSLYQLENYCN

#### **(2) T20 Peptide:**

YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF

#### **(3) Interferon Alpha 2B:**

MALTFALLVALLVLSCSSCSVGC DLPQTHSLGSRRTLMLLAQMRRISLF SCLKDRHDFGFPQE EFGNQF  
QKAETIPVLHEMIQQIFNL FSTKDSSAAWDETLLDKFYTELYQQ LNDLEACVIQGVGTETPLMKEDSIL  
AVRKYFQ RITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLSKE

#### **(4) Tobacco Etch Virus Protease:**

GESLFGKPRDYNPISS TICHLTNESDGHTTSLYGIGFGPFIITNKHLFRRNNGTLLVQSLHGVFKVKNTT  
TLQQHLIDGRDIIIRMPKDFPFPQKLKFREPQREERICLVTTNFQTKSMSSMVSDTSCTFPSSDGIFW  
KHWIQTKDGQCGSPLVSTRDGFIVGIHSASNFTNTNNYFTSVPKNFMELLTNQEAQQWVSGWRLNADSVL  
WGGHKVFMSKPEEPFQPVKEATQLMNELVYSQ

#### **(5) Small Heterodimer Partner Orphan Receptor:**

MSTSQPGACPCQGAASRPAILYALLSSSLKAVPRPRSRCLCRQHRPVQLCAPHRTCREALDVLAKTVAFL  
RNLPSFWQLPPQDQRRLLQGCWGPLFLLGLAQDAVTFEVAEAPVPSILKKILLEEPSSSGSGQLPDRPQ  
PSLAAVQWLQCCLESFWSLELSPKEYACLKGTILFNPDVPGLOAASHIGHLQEAHWLCEVLEPWCPAA  
QGRLTRVLLTASTLKS IPTSLLGDLFFRPIIGDVDIAGLLGDMLLLR

#### **(6) Androgen Receptor Ligand Binding Domain:**

MHIEGYECQPIFLNVLEAIEPGVVCAGHDNNQPDSFAALLSSLNELGERQLVHVVKWAKALPGFRNLHVD  
DQMAVIQYSWMGLMVFAMGWSFTNVNSRMLYFAPDLVFNEYRMHKSRMYSQCVRMRHLSQEFGLQITP  
QEFLCMKALLLFSIIPVDGLKNQKFFDELRMNYIKELDRIIACKRKNPTSCSRRFYQLTKLLDSVQPIAR  
ELHQFTFDLLIKSHMVSVD FPEMMAEIIISVQVPKILSGKVKPIYFHTQ

#### **(7) Glucocorticoid Receptor Ligand Binding Domain:**

MIQQATTGVSQETSENP GDKTIVPATLPQLTPTLVSLLEVIEPEVLYAGYDSSVPDSTWRIMTTLNMLGG  
RQVIAAVKWAKAIPGFRNLHLD DQMTLLQYSWMSLMAFALGWSYRQSSANLLCFAPDLIINEQRM TLPD  
MYDQCKHMLYVSSELHRLQVS YEEYLCMKTLLLLSSVPKDGLKSQELFDEIRMTYIKELGKAI VKREGNS  
SQNWQRFYQLTKLLDSMHEVVENLLNYCFQTFDKTMSIEFPEMLAEIITNQIPKYSNGNIKKLLFHQK

#### **(8) Estrogen Receptor Ligand Binding Domain:**

MSKKNSLALSLTADQMVSALLDAEPPILYSEYDPTRPFSEASMMGLLTNLADRELVHMINWAKRVPGFVD  
LTLHDQVHLLCAWLEILMIGLVWRSMHEHPGKLLFAPNLLLDNRNQKGCVEGMVEIFDMLLATSSRFRMMN  
LQGEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMKAGLTLOQQHQRLAQLLL  
ILSHIRHMSNKGMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVEETDQSHLATAGSTSSSHSL  
QKYIITGEAEGFPATV

(9) G Protein Alpha Q:

MTLESIMACCLSEEAKEARRINDEIERQLRRDKRDARRELKLLLLGTGESGKSTFIKQMRIIHGSGYSDE  
DKRGFTKLVIQNI FTAMQAMIRAMD TLKIPYKYEHNAHAQLVREVDVEKVS AFENPYVDAIKSLWNDPG  
IQECYDRRREYQLSDSTKYLLNDLDRVADPAYLPTQQDVLVRVRVPTTGII EYPFDLQSVIFRMVDVGGQR  
SERRKWIHCFENVTSIMFLVALSEYDQVLVESDNENRMEESKALFRTIITYPWFQNSSVILFLNKKDLLE  
EKIMYSHLVDYFPEYDGPQRDAQAAREFILKMFVDLNPDS DKINYSHTCATDTENIRFVFAAVKDTILQ  
LNLKEYNLV

(10) 1-Deoxy-D-Xylulose 5-Phosphate Reductoisomerase Peptide:

MKQLTILGSTGSIGCSTLDVVRHNPEHFRVVALVAGKNVTRMVEQCLEFS PRYAVMDDEASAKLLKTMLO  
QQGSRTVLSGQQAACDMAALEDVDQVMAAIVGAAGLLPTLAAIRAGKTILLANKESLVTCGRLFMDAVK  
QSKAQLLPVDSEHNAIFQSLPQPIQHNLGYADLEQNGVVSILLTGSGGPFRETPLRDLATMTPDQACRHP  
NWSMGRKISVDSATMMNKGLEYIEARWLFNASASQMEVLIHPQS VIHSMVRYQDGSVLAQLGEPDMRTPI  
AHTMAWPNRVNSGVKPLDFCKLSALTFAAPDYDRYPCLKLAMEAFEQQAATTALNAANEITVAAFLAQQ  
IRFTDIAALNLSVLEKMDMREPQCVDVLSVDASAREVARKEVMRLASPV

(11) G Protein Alpha S:

MGCLGNSKTEDQRNEEKAQREANKKIEKQLQKDKQVYRATHRLLLLGAGESGKSTIVKQMRILHVNGFNG  
DSEKATKVQDIKNNLKEAIE TIVAAMSNLVPVELANPENQFRVDYILSVMNVPDFDFPPEFYEHAKALW  
EDEGVRACYERSNEYQLIDCAQYFLDKIDVIKQADYVPSDQDLLRCRVLTSGIFETKFQVDKVNFMFDV  
GGQDERRKWIQCFNDVTAIIFVVASSSYNMVIREDNQTNRLQEALNLFKSIWNNRWLRTISVILFLNKQ  
DLAELKVLGKSKIEDYFPEFARYTTPEDATPEPGEDPRVTRAKYFIRDEFRLISTASGDGRHYCYPHFT  
CAVDTENIRRVFNDCRDI IQRMHLRQYELL

**(B). Phase Transition Protein Sequences**

(1) ELP1-60 (PflM1-Bgl1):

GVGVPGVGPVGGGVPGAGVPGVGPVGVGPVGPVGGGVPGAGVPGGVPGVGPVGVGPVGGGVPGAGVP  
GVGVPGVGPVGVGPVGGGVPGAGVPGGVPGVGPVGVGPVGGGVPGAGVPGVGPVGVGPVGVGPVGGGV  
GAGVPGGVPGVGPVGVGPVGGGVPGAGVPGVGPVGVGPVGVGPVGGGVPGAGVPGGVPGVGPVGVGP  
GGGVPGAGVPGVGPVGVGPVGVGPVGGGVPGAGVPGGVPGVGPVGVGPVGGGVPGAGVPGVGPVGVGP  
GVGVPGGVPGAGVPGGVPG

(2) ELP4-60 (PflM1-Bgl1):

GVGVPGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGP  
GVGVPGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGP  
GVGVPGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGPVGVGP





- (2) Containing enterokinase protease (EK) cleavage site K/G:

- (4) Containing tobacco etch virus protease (TEV) cleavage site Q/Y:

- (5) Containing tobacco etch virus (TEV) protease cleavage site Q/G:

- ### (D) Fusion Protein Sequences<sup>1</sup>

- (1) FP#1: (pET32a-SD15-ELP4-60-EK-Insulin A peptide):

[illegible]

- (2) FP#2: (pET32a-SD15-ELP1-90-EK-Insulin A peptide):

MGGPGVGVPGVGVPGGGVPAGAGVPGVGVPGVGVPGVGVPGGGVPAGAGVPGGGVPGVGVPGVGVPGGGVP  
AGVPGVGVPGVGVPGVGVPGGGVPAGAGVPGGGVPGVGVPGVGVPGGGVPAGAGVPGVGVPGVGVPGVGVP  
GGVPAGAGVPGGGVPGVGVPGVGVPGGGVPAGAGVPGVGVPGVGVPGVGVPGGGVPAGAGVPGGGVPGVGVP  
VGVPGGGVPAGAGVPGVGVPGVGVPGVGVPGGGVPAGAGVPGGGVPGVGVPGVGVPGGGVPAGAGVPGVGVP  
VGVPGVGVPGGGVPAGAGVPGGGVPGVGVPGVGVPGGGVPAGAGVPGVGVPGVGVPGVGVPGGGVPAGAGVP

<sup>1</sup> with ELP protein underlined, target protein bold and underlined, spacer marked by gray, and cleavage site indicated by a forward slash.

(3) **FP#3: (pET32a-SD15-ELP4-120-EK-Insulin A peptide):**

(4) FP#4: (pET32a-SD15-ELP1-180-EK-Insulin A peptide):

(5) FP#5: (pET15b-ELP4-60-EK-T20 peptide):

(6) FP#6: (pET17b-ELP1-90-EK-T20 peptide):

25

(7) **FP#7: (pET15b-ELP4-120-EK-T20 peptide):**

(8) FP#8: (pET17b-ELP4-60-Throm-T20 peptide):

(9) FP#9: (pET17b-ELP1-90-Throm-T20 peptide):

(10) FP#10: (pET17b-ELP4-120-Throm-T20 peptide):

[illegible]



(16) FP#16: (pET17b-ELP4-120-TEV(Q/Y)-T20 peptide):

(17) FP#17: (pET32a-SD11-ELP1-90-throm-Interferon Alpha 2B):

(18) FP#18: (pET15b-SD5-ELP4-60-throm-Tobacco etch virus protease):

(19) FP#19: (pET15b-SD5-ELP1-90-throm-Tobacco etch virus protease):

28

(20) FP#20: (pET15b-SD5-ELP4-120-throm-Tobacco etch virus protease):

(21) FP#21: (pET15b-SD5-ELP1-180-throm-Tobacco etch virus protease):

(22) FP#22: (pET15b-SD3-ELP1-90-throm-Small Heterodimer partner orphan receptor):

(23) FP#23: (pET15b-SD3-ELP1-90-throm-Androgen receptor ligand binding domain):

(24) FP#24: (pET15b-SD3-ELP1-180-throm-Androgen receptor ligand binding domain):

(25) FP#25: (pET15b-SD3-ELP1-90-throm-Glucocorticoid receptor ligand binding domain):

(26) FP#26: (pET15b-SD3-ELP1-60-throm-Estrogen receptor ligand binding domain):

(27) FP#27: (pET15b-SD5-ELP1-90-throm-Estrogen receptor ligand binding domain):

(28) FP#28: (pET15b-SD5-ELP1-180-throm-Estrogen receptor ligand binding domain):

31



VPGVGVPGGGVPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGVGV  
 VPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGVGVPGVGVPGVGVPGGGVPGAG  
 VPGGGVPGVGVPGVGVPGGGVPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGG  
 VPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGVGVPGVGVPGVGV  
 VPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGWP  
SSGLVPR/GSPGTSGGGGHMSKNSLALSLTADQMVSALLDAEPPILYSEYDPTRPFSEASMMGLLTNL  
ADRELVHMINWAKRVPGFVDLTLHDQVHLLECAWLEILMIGLVWRSMEHPGKLLFAPNLLLDNRNQKCE  
GMVEIFDMLLATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHL  
MAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEHLYSMKCKNVPPLYDLLEMLDAHRLHAPTSRGGAS  
VEETDQSHLATAGSTSSHSLQKYYITGEAEGFPATV

(29) FP#29: (pET15b-SD6-ELP1-90-TEV-Estrogen receptor ligand binding domain):

MRALMGPGVGVPGVGVPGGGVPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGG  
 VPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGVGVPGVGVPGVGV  
 VPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGV  
 VPGVGVPGGGVPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGVGV  
 VPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGVGVPGVGVPGVGVPGGGVPGAG  
 VPGGGVPGVGVPGVGVPGGGVPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGG  
 VPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGV  
 VPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGV  
WPSSGGDYDIPTTENLYEQ/GAHMSKNSLALS  
LTADQMVSALLDAEPPILYSEYDPTRPFSEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLL  
ECAWLEILMIGLVWRSMEHPGKLLFAPNLLLDNRNQKCEGMVEIFDMLLATSSRFRMMNLQGEEFVCLK  
SIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSN  
KGMEHLYSMKCKNVPPLYDLLEMLDAHRLHAPTSRGGASVEETDQSHLATAGSTSSHSLQKYYITGEAE  
GFPATV

(30) FP#30: (pET15b-SD1-ELP1-90-throm-G protein alpha Q):

MRALMGPGVGVPGVGVPGGGVPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGG  
 VPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGVGVPGVGVPGVGV  
 VPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGV  
 VPGVGVPGGGVPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGVGV  
 VPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGVGVPGVGVPGVGVPGGGVPGAG  
 VPGGGVPGVGVPGVGVPGGGVPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGG  
 VPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGV  
WPSSGGGSIGPLVPR/GSHSMGLNDIFEAQKI  
EWHEHMPMALEMTLESIMACCLSEEAKEARRINDEIERQLRRDKRDARRELKLLLLGTGESGKSTFIKQM  
RIIHSGYSDEDKRGFTKLVYQNIFTAMQAMIRAMDTLKIPYKYEHNKAHAQLVREVDVEKVSAFENPYV  
DAIKSLWNDPGIQECYDRRREYQLSDSTKYYLNDLDRVADPAYLPTQQDVLRVRVPTTGIIEYPFDLQSV  
IFRMVDVGGQRSERRKWIHCFENVTSIMFLVALSEYDQVLVEDNENRMEESKALFRTIITYPWFQNSSV  
ILFLNKKDLLEEKIMYSHLDYFPEYDGPQORDAQAAREFILKMFVDLNPDSDKINYSHFTCATDTENIRF  
VFAAVKDTILQLNLKEYNLV

(31) FP#31: (pET15b-SD1-ELP1-180-throm-G protein alpha Q):

MRALMGPGVGVPGVGVPGGGVPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGG  
 VPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGVGVPGVGVPGVGV  
 VPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGV  
 VPGVGVPGGGVPGAGVPGVGVPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGVGV  
 VPGVGVPGVGVPGGGVPGAGVPGGGVPGVGVPGVGVPGGGVPGAGVPGVGVPGVGVPGVGVPGGGVPGAG

VPGGGVPGVGPVGVPVPGGGVPGAGVPGVGPVGVPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGG  
 VPGAGVPGVGPVGVPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVG  
 VPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVG  
 VPGVGPVPGGGVPGAGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGAG  
 VPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGG  
 VPGAGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGG  
 VPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGW  
 SSGGGSIGPLVPR/GSHSMGLNDIFEAQKIEWHEHMPMALEMTLESIMACCLSEEAKEARRINDEIERQL  
 RRDKRARRELKLLLLGTGESGKSTFIKQMRIIHGSGYSDKRGFTKLVIYQNIIFTAMQAMIRAMDTLKI  
 PYKYEHNKAHAQLVREVDVEKVSFAFENPYVDAIKSLWNDPGIQECYDRRREYQLSDSTKYLLNDLDRVAD  
 PAYLPTQQDVLVRVPTTGIIEYPFDLQSVIFRMVDVGGQRSEKRWIHCFFENVTSIMFLValseyDQVL  
 VESDNENRMEEskalfRTIITYPWFQNSSVILFLNKKDLLEEKIMYSHLVDYFPEYDGPQRDAQAAREFI  
 LKMFVDLNPDSKINYSHFTCATDTENIRFVFAAVKDTILQLNLKEYNLV

(32) FP#32: (pET15b-SD3-ELP1-60-throm-1-Deoxy-D-Xylulose 5-Phosphate Reductoisomerase Peptide):

MRALMGPGVGPVGVPVPGGGVPGAGVPGVGPVGVPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGG  
 VPGAGVPGVGPVGVPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVG  
 VPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVG  
 VPGVGPVPGGGVPGAGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGV  
 VPGVGPVPGVGPVPGGGVPGAGVPGGGVPGWPSGGGGGSIGPLVPR/GSHMKQLTILGSTGSIGCSTLDVV  
 RHNPEHFRVVALVAGKNVTRMVEQCLEFSPRYAVMDDEASAKLLKTMLQQQGSRTTEVLSGQQAACDMAAL  
 EDVDQVMAAIVGAAGLLPTLAAIRAGKTILLANKESLVTGRLFMDDAVKQSKAQLLPVDSEHNAIFQSLP  
 QPIQHNLGYADLEQNGVVSILLTGSGGPFRETPLRDLATMTPDQACRHPNWSMGRKI SVDSATMMNKGLE  
 YIEARWLFNASASQMEVLIHPQSVIHSMVRYQDGSVLAQLGEPDMRTPIAHTMAWPNRVNSGVKPLDFCK  
 LSALTFAAPDYDRYPCLKLAMEAFEQQAATTALNAANEITVAFLAQQIRFTDIAALNLSVLEKMDMRE  
 PQCVDVLSVDASAREVARKEVMRLASPV

(33) FP#33: (pET15b-SD5-ELP1-90-throm-1-Deoxy-D-Xylulose 5-Phosphate Reductoisomerase Peptide):

MRALMGPGVGPVGVPVPGGGVPGAGVPGVGPVGVPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGG  
 VPGAGVPGVGPVGVPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVG  
 VPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVG  
 VPGVGPVPGGGVPGAGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGV  
 VPGVGPVPGVGPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAG  
 VPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGGVPGAGVPGGGVPGVGPVGVPVPGGG  
 VPGAGVPGVGPVGVPVPGGGVPGAGVPGGGVPGWPSGLVPR/GSPGISGGGGGHMKQLTILGST  
 GSIGCSTLDVVRHNPEHFRVVALVAGKNVTRMVEQCLEFSPRYAVMDDEASAKLLKTMLQQQGSRTTEVLS  
 GQQAACDMAALEVDQVMAAIVGAAGLLPTLAAIRAGKTILLANKESLVTGRLFMDDAVKQSKAQLLPVD  
 SEHNAIFQSLPQPIQHNLGYADLEQNGVVSILLTGSGGPFRETPLRDLATMTPDQACRHPNWSMGRKISV  
 DSATMMNKGLE YIEARWLFNASASQMEVLIHPQSVIHSMVRYQDGSVLAQLGEPDMRTPIAHTMAWPNRV  
 NSGVKPLDFCKLSALTFAAPDYDRYPCLKLAMEAFEQQAATTALNAANEITVAFLAQQIRFTDIAALN  
 LSVLEKMDMREPQCVDVLSVDASAREVARKEVMRLASPV

(34) FP#34: (pET15b-SD5-ELP1-180-throm-1-Deoxy-D-Xylulose 5-Phosphate Reductoisomerase Peptide):

[illegible]

MRALMGPGVGVPGVGVPGGGVPAGVPGVGPVGVPVGVPGGGVPAGVPGGGVPGVGPVGVPVG  
VPGAGVPGVGVPGVGVPGVGVPGGGVPAGVPGGGVPGVGPVGVPVGVPGGGVPAGVPGVGVPVGVPVG  
VPGGGVPAGVPGGGVPGVGVPGVGVPGGGVPAGVPGVGVPGVGVPGVGVPGGGVPAGVPGGGVPGV  
VPGVGVPGGGVPAGVPGVGVPGVGVPGVGVPGGGVPAGVPGGGVPGVGPVGVPVGVPGGGVPAGVPGV  
VPGVGVPGVGVPGGGVPAGVPGGGVPGVGVPGVGVPGGGVPAGVPGVGVPGVGVPGVGVPGGGVPAG  
VPGGGVPGVGVPGVGVPGGGVPAGVPGVGVPGVGVPGVGVPGGGVPAGVPGGGVPGVGVPGVGVPGG  
VPGAGVPGVGVPGVGVPGVGVPGGGVPAGVPGGGVPGVSSGDYDIPPTTENLYFQ/GAHMKQLTILGST  
GSIGCSTLDVVRHNPEHFRVALVAGKNVTRMVEQCLEFSPIRYAVMDDEASAKLLKTMLOOQGSRTVL  
GQQAACDMAALEDVDQVMAAIVGAAGLLPTLAAIRAGKTILLANKESLVTCGRLFMDAVKQSKAQLLPVD  
SEHNAIFQS L P Q I Q H N L G Y A D L E Q N G V S I L L T G S G G P F R E T P L R D L A T M T P D Q A C R H P N W S M G R K I S V  
D S A T M M N K G L E Y I E A R W L F N A S A S Q M E V L I H P Q S V I H S M V R Y Q D G S V L A Q L G E P D M R T P I A H T M A W P N R V  
N S G V K P L D F C K L S A L T F A A P D Y D R Y P C L K L A M E A F E Q Q A A T T A L N A A N E I T V A A F L A Q Q I R F T D I A A L N  
L S V L E K M D M R E P Q C V D D V L S V D A S A R E V A R K E V M R L A S P V

[illegible]

KVLAGKSKIEDYFPEFARYTTPEDATPEPGEDPRVTRAKYFIRDEFLRISTASGDGRHYCYPHFTCAVD  
TENIRRVFNDCRDI IQRMHLRQYELL